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Comparison of microstructure of pheromone transfer particles (PTPs) in the genus *Tirumala* (Lepidoptera, Nymphalidae, Danainae)

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Abstract Pheromone transfer particles (PTPs) found in the male alar pouch of the genus *Tirumala* serve to make females recognize their mates and there is a possibility that the chemical composition of the pheromone is species specific. In order to evaluate this possibility, the microstructure of PTPs for seven species of *Tirumala* was compared using a scanning electron microscope. PTPs of *T. gautama*, *choaspes*, *ishmoides*, *septentrionis* and *hamata* are, though slightly different in shape, almost rounded. On the other hand, those of *T. limniace* and *T. petiverana* are characteristically polyhydric in shape and a little smaller in size. There is a possibility that the characteristic microstructure of PTPs in individual *Tirumala* species reflects the clear sexual isolation.

Key words *Tirumala*, Danainae, PTPs, microstructure, SEM, courtship behavior, sexual isolation, species specificity.

Introduction

The genus *Tirumala* in the Danainae consists of 9 species, with 2 species distributed in Africa and 7 species in the Indo-Australian region (Ackery & Vane-Wright, 1984). The genus is known to have alar organs, termed alar pouches on the male hindwing cell Cu_{1b}, which is characteristic for *Tirumala*. Alar organs of this peculiar form are not found in other danaine butterflies. Inside the alar pouch, there are scale sockets and disk-shaped sockets on the surface, and, from these disk-shaped sockets, characteristic long and narrow scales (called cushion scales) grow (Fig. 1). These cushion scales are reduced to particles after a certain period of time, generating a large number of pheromone transfer particles (PTPs) (Fig. 2).

PTPs (commonly seen in Danainae) are transferred by adhering to the tip of the abdominal hairpencil and play a role in females to accept copulation by scattering pheromones in front of them or by directly sticking to their antennae during courtship behavior. PTPs are smell-producing materials which induce a female to recognize her partner for copulation precisely. So there is a possibility that the morphology of PTPs is specific to species, similarly to the chemical composition of the pheromone.

Although Boppré & Vane-Wright (1989) examined the microstructure of PTPs of *Tirumala petiverana* by SEM, characteristics of PTPs have not been reported so far for the whole *Tirumala* genus. For this reason, we compared the morphology of 7 species of *Tirumala* PTPs using a scanning electron microscope.

Materials and methods

For observation of PTP microstructure, a part of the pocket-shaped alar organs was taken from the male hindwing, and opened so that the inside could be seen; the dissected organs were glued onto a metal stub and gold-coated three times for four minutes each using a JOEL JSM-35 ion sputtering system.

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Subsequently, the inside of the alar pouch was observed by using a SEM JEOL JSM-5600LV at 15 kV voltage and at a magnification of 800×, 1,000×, 2,500×, 10,000× and 15,000×.

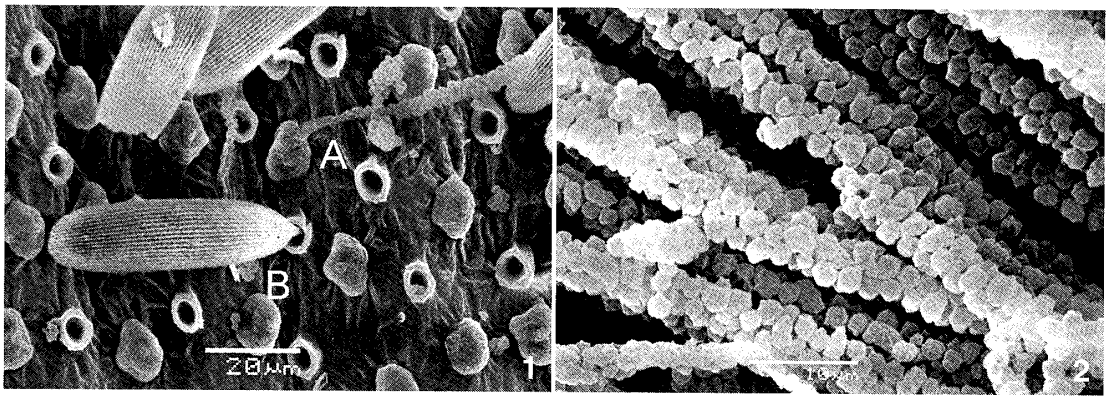
Materials used are summarized in Table 1. The specimens of *T. gautama* used were borrowed from the Research Institute of Evolutionary Biology, Tokyo; for the other species, dried specimens in the collection of the Graduate School of Social and Cultural Studies, Kyushu University were used.

Results

According to SEM observation, though slightly different in shape, PTPs of 5 species (*T. gautama*, *choaspes*, *ishmoides*, *septentrionis* and *hamata*) are almost spherical (Figs 3–7). Characteristic wrinkles were seen in *T. gautama* (Fig. 3) and *T. choaspes* (Fig. 4), on the surface. PTPs of *T. limniace* (Figs 8–9) and *T. petiverana* (Fig. 10) are characteristically

Table 1. Table 1 Used materials for observation of PTPs microstructure of male *Tirumala*.

species/subspecies	locality	date of capture	number
T. gautama gautama	Hainan	December 1980	2
T. choaspes choaspes	S. Sulawesi	August 2002–March 2004	5
T. ishmoides ishmoides	Buton	?	1
T. septentrionis septentrionis	Vietnam	June 1997	1
Ditto	Hainan	March 2004	2
Ditto	N. Thailand	July 2005	2
T. limniace limniace	Thailand	August 2001	1
Ditto	N. India	June 1992	2
Ditto	Taiwan	August 1952	1
T. l. orestilla	Luzon	December 1969	4
T. l. makassara	S. Sulawesi	August 2003	1
T. hamata leucoptera	Irian Jaya	February 2003	4
T. h. orientalis	Luzon	December 1969	3
T. h. tibura	Bohol	August 1998	1
T. h. paryadres	Moa	January 2003	1
T. petiverana	Kenya	November 2004	3



Figs 1–2. Microstructure of PTPs of male *Tirumala septentrionis*. 1. Interior of pouch showing cushion (A) and pouch (B) scales (×800). 2. Details of particle-generating cushion scales (×2,500).

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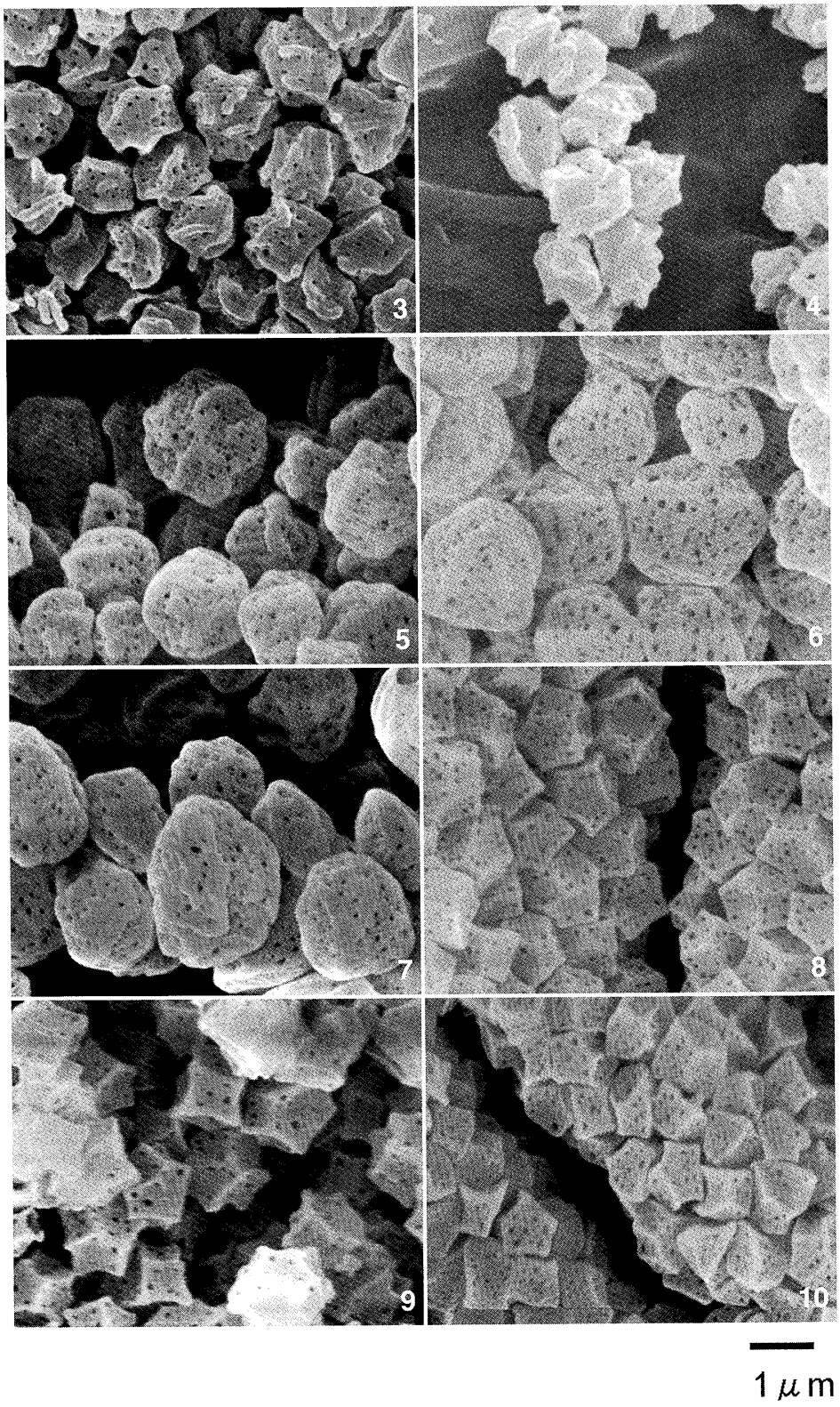

Figs 3–10. Microstructure of PTPs of male *Tirumala* spp. (× 15,000). 3. *T. gautame gautama*. 4. *T. choaspes choaspes*. 5. *T. ishmoides ishmoides*. 6. *T. septentrionis septentrionis*. 7. *T. hamata orientalis*. 8. *T. limniace orestilla*. 9. *T. limniace limniace*. 10. *T. petiverana*.

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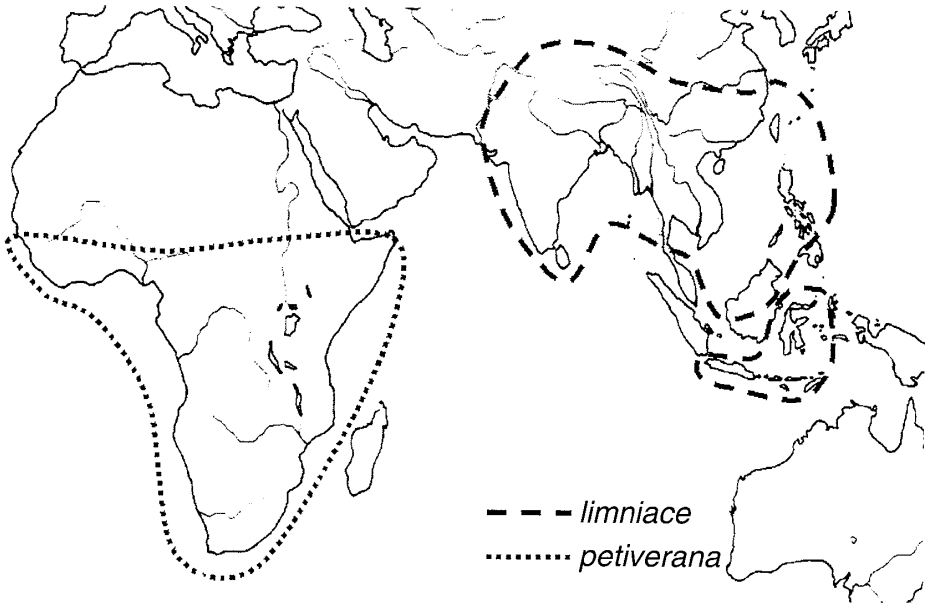

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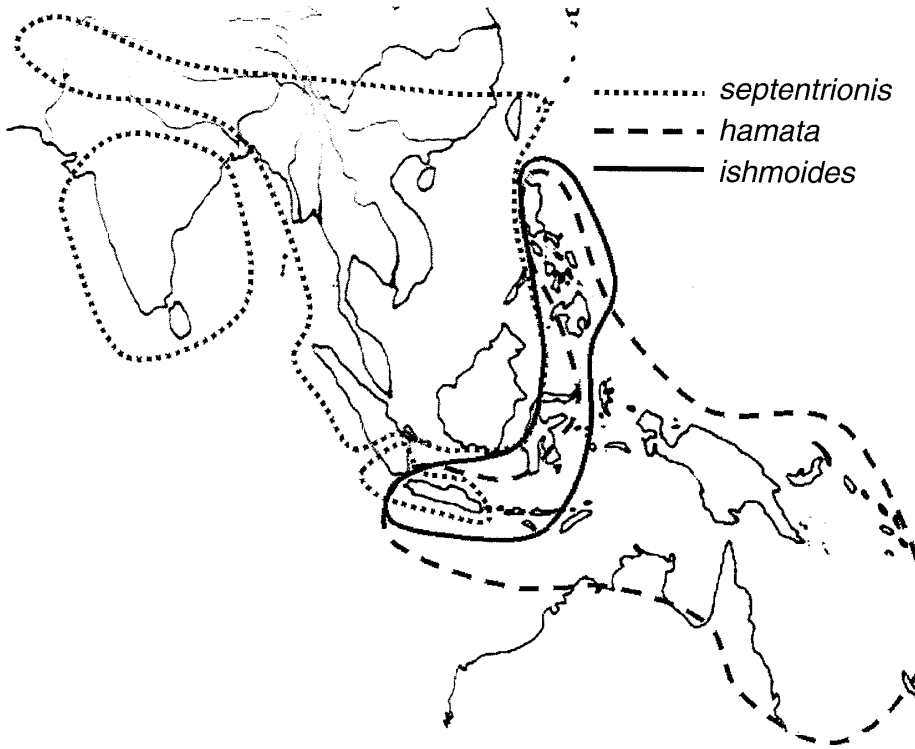

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Figs 11–12. Distribution maps of some *Tirumala* species 11. *T. limniace* and *T. petiverana*. 12. *T. septentrionis*, *T. hamata* and *T. ishmoides*.

polyhedral and closely resemble each other.

Particles measure 1.2–1.5 μm in diameter for *T. gautama* and *T. choaspes* and 1.5–2.0 μm for *T. ishmoides*, *septentrionis* and *hamata*. Those of *T. limniace* and *T. petiverana* are a little smaller, measuring 1.0–1.5 μm in diameter.

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Discussion

There is a possibility that the characteristic morphology of PTPs of *T. limniace* reflects the sexual isolation from other *Tirumala* species as seen in species specificity of pheromone. It is considered that such the sexual isolation mechanism plays an important role and its specificity is also reflected in the morphology of PTPs, because *T. limniace* is a common species with a wide distribution and thereby often flies together with other congeners such as *T. septentrionis* and *T. hamata*. On the other hand, the similarity of PTPs morphology between *T. limniace* from tropical Asia and *T. petiverana* from Africa (Fig. 2) shows that the two species are closely related (probably vicariant species) as evidenced by the fact that they had been treated as different subspecies of the same species from Talbot (1943) to D'Abrera (1980).

Moreover, the morphology of PTPs is very closely similar not only between *T. limniace* and *T. petiverana* but also among three species (*T. ishmoides*, *septentrionis* and *hamata* (Fig. 1), especially the latter two). As suggested by the fact that *T. septentrionis* had been treated as a subspecies of *hamata* from Talbot (1943) to Kawazoé & Wakabayashi (1977) and others, the morphology of the male genitalia and other characters is quite similar between the two species. The resemblance of these morphological characters, which tend to be species specific, suggests a close relationship between *T. septentrionis* and *T. hamata*.

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摘　　要

Tirumara 属内のフェロモン運搬粒子 PTPs の微細構造の比較 (橋本　恵・矢田　脩)

コモンマダラ属 *Tirumala* の性標内にあるフェロモン運搬粒子 PTPs は, 雌に交尾相手を正確に認識させるものであり, フェロモンの化学成分とともに, この PTPs の形態にも種特異性が認められる可能性がある. そこで, 走査型電子顕微鏡を用いて, *Tirumala* 属 7 種の PTPs の形態を観察し比較した.

その結果, *T. gautama*, *choaspes*, *ishmoides*, *septentrionis*, *hamata* のフェロモン運搬粒子 (PTPs) は, 形状に若干の違いはあるものの, どれもおおむね丸い形状をしている. 一方, *T. limniace* とアフリカに分布する *T. petiverana* の PTPs は, 特有の多面体をしており, 粒子の大きさは若干小さい. *T. limniace* の特異

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なPTPsの形態は, フェロモンの種特異性など*Tirumala*属の他の種との明確な性的隔離を反映している可能性が高いと考えられる. また, *Tirumala*属の中で熱帯アジア産*T. limniace*とアフリカ産*T. petiverana*のPTPsの形態が相互に類似することは, かつて両者が同種に扱われていたように, 両者は互いに極めて近縁の種であることを支持する証拠となる.

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